Reference: *Biol. Bull.* **187**: 16–22. (August, 1994)

# Muscle and Nerve Terminal Fine Structure of a Primitive Crustacean, the Cephalocarid *Hutchinsoniella macracantha*

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**Abstract.** Abdominal muscles of the cephalocarid *Hutchinsoniella macracantha* resemble the striated muscle fibers of other crustaceans, having regularly aligned sarcomeres that average 5 μm in length; thick, wavy Z-lines; and orbits of eight thin filaments surrounding a thick filament. However, unlike most crustacean muscle fibers, the cephalocarid muscle fibers are not subdivided into myofibrils by elaboration of the longitudinally oriented sarcoplasmic reticulum. Consequently, elements of the transverse tubule and sarcoplasmic reticulum in the form of triads occur scattered over the entire fiber. Motor innervation is by means of scattered nerve terminals, populated with round synaptic vesicles, indicative of excitatory axons. By lacking myofibrils, the cephalocarid and ostracod muscle represents a much simpler condition than the myofibril-rich muscles of the other crustacean classes and signifies a primitive condition in its resemblance to the onycophoran muscle.

## Introduction

Members of the class Cephalocarida are the most primitive living crustaceans, on the basis of comparative studies of the external morphology, skeletomusculature, larval development, and behavior of the best-known species, *Hutchinsoniella macracantha* (Sanders, 1957, 1963; Hessler, 1964, 1992; Hessler and Newman, 1975). Studies of the nervous (Elofsson and Hessler, 1990, 1991, 1992) and digestive (Elofsson *et al.*, 1992) systems reveal fundamental apomorphies that indicate an ancient split from the rest of the Crustacea. Thus although cephalocarids can tell little about the interrelationships within Crustacea, they may tell much about the roots of the crustacean radiation. For this reason, it is important to know as much as we can about the anatomy of this small taxon.

Received 9 November 1993; accepted 19 May 1994.

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The gross anatomy of the musculature of *H. macracantha* has been analyzed in detail (Hessler, 1964), but its fine structure has never been described. This is the subject of the present study. We focus on the abdominal musculature because of its simplicity.

The cephalocarid body is composed of cephalon, thorax, and abdomen. The cephalon and thorax are dorsoventrally compressed and bear appendages. As a result, the musculature of these tagmata is complicated. The abdomen is virtually a limbless, articulated cylinder capable of strong flexion in all directions; only the telson bears a terminal caudal furca. Hence the organization of the muscles in the abdomen is much simpler than that of the thorax, consisting of paired sheaths of dorsal and ventral longitudinal muscles that line the exoskeleton and insert upon it at the anterior end of each segment (Hessler, 1964).

## Materials and Methods

Adults of *Hutchinsoniella macracantha* were collected from the silt-clay bottom of Buzzards Bay near Woods Hole, Massachusetts, and prepared for electron microscopy. The tiny elongate animals (about 3 mm) were cut transversely into two or three pieces and fixed for 1–2 h in 3% glutaraldehyde and 0.7 *M* NaCl in 0.1 *M* sodium cacodylate buffer (pH 7.4). After a brief rinse in buffer, the tissue was postfixed in 2% osmium tetroxide for 1 h, washed in buffer, dehydrated in ethanol, cleared in propylene oxide, and embedded in plastic. Thin (80–100 nm) sections were obtained with a diamond knife, mounted on formvar-coated single-slot grids, and stained with uranyl acetate and lead citrate. The tissue was examined with a Siemens 102 or Zeiss 9A electron microscope. The abdominal musculature from five animals was examined.

## Results

### *Muscle*

The muscle bundle in each segment is made up of a single layer of roughly elliptical fibers (Fig. 1A). Each fiber is defined by a distinct sarcolemma and is tightly packed with myofilaments. Mitochondria are found along the periphery of the fiber and are not seen within the body of the fiber amongst the myofilaments. Also along the periphery of the muscle sheath are muscle nuclei and motor nerve terminals. Individual fibers have numerous folds and invaginations of the sarolemmal membrane along their length, and this gives them a complex outline typical of crustacean muscle (Atwood, 1973).

The myofilaments are arranged into distinct sarcomeres defined by adjacent Z-lines that enclose I-bands on either side of a central A-band (Fig. 1C). The Z-lines are thick and wavy and somewhat regularly aligned, as are the sarcomeres. Measurements of individual sarcomeres from three animals revealed a range of 3.6 to 7.9 $\mu$m and a mean (SD) value of 5.1 $\mu$m ($\pm$0.95, $n = 45$). To ensure that these differences are not due to differences in overlap of thick and thin filaments, individual A-bands were measured because these are less subject to change. The A-bands showed a trend similar to that of the sarcomeres, with a range of 3.2 to 4.8 $\mu$m and a mean (SD) value of 3.7 $\mu$m ($\pm$0.38, $n = 45$). In cross-sectional view (Fig. 1B), the thick filament has an orbit of 6–10 thin filaments, for a mean (SD) of 8.3 ($\pm$1.1, $n = 87$).

The tubule system in these cephalocarid muscles consists of the transverse tubules (t-tubules), which invaginate from the sarcolemma at the level of the I-band and Z-line, with branches in the A-band region forming part of a triad (Fig. 2A). The other system of tubules, *viz.*, the sarcoplasmic reticulum, is relatively simple, consisting of one or two longitudinally running tubules (Fig. 1C). Where these are juxtaposed on either side of the longitudinal elements of the t-tubule, a triad arrangement of tubules is seen in cross-section (Fig. 1B). Triads are restricted to the A-band region, where they occur scattered over the entire fiber (Fig. 3). The sarcoplasmic reticulum therefore does not form an elaborate network of tubules that subdivides the muscle fiber into myofibrils. Indeed, the absence of myofibrils is the most striking feature of the cephalocarid muscle fiber (Figs. 3, 4A).

### *Neuromuscular terminals*

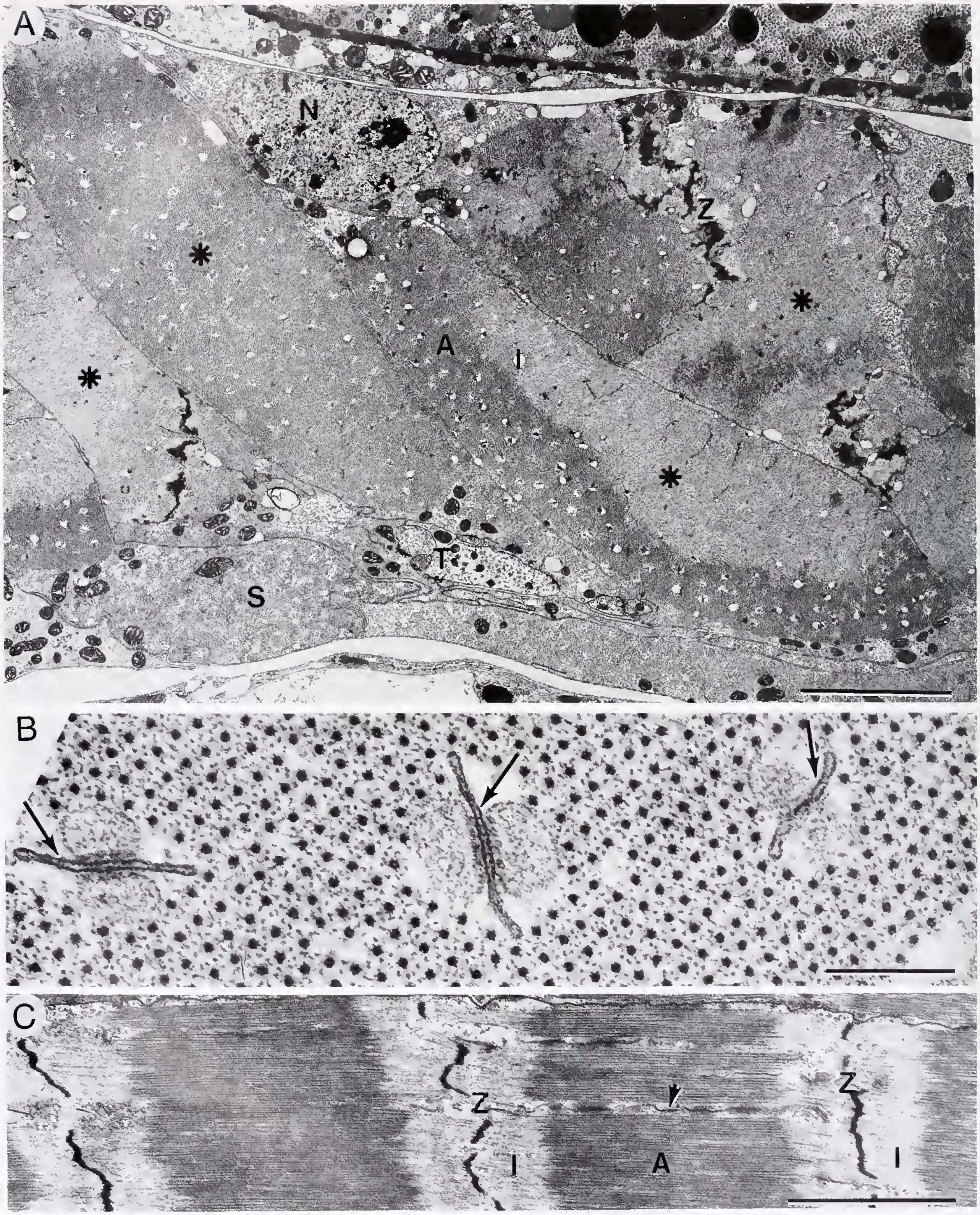
Motor innervation to the muscle group usually consists of a cluster of nerve terminals along the periphery of the muscle (Fig. 1A). These nerve terminals are located adjacent to the myofilaments on small islands of the muscle granular sarcoplasm that have relatively few tubules (Fig. 2B). The motor nerve terminals are characteristically populated with clear synaptic vesicles and make synaptic contact with the muscle granular sarcoplasm. At these synaptic contacts the membranes are densely stained and a dense body is found on the presynaptic membrane, denoting an active site for transmitter release. Mitochondria are seen in these terminals, but dense-core vesicles are not. The clear synaptic vesicles surveyed in more than 50 nerve terminals in five separate animals are spherical, suggesting that these are excitatory terminals because with aldehyde fixation the synaptic vesicles assume a characteristic shape—spherical for excitatory and elliptical for inhibitory axons (Tisdale and Nakajima, 1976).

## Discussion

### *Muscle*

The fine structure of the abdominal muscles of the cephalocarid *H. macracantha* is typical of crustacean striated muscle in that the myofilaments are highly organized in the transverse plane into sarcomeres (Atwood, 1973; Govind and Atwood, 1982). Also typical of the abdominal muscle in tailed decapods such as crayfish and lobsters is the differentiation into fast and slow fibers based on sarcomere length; short (2–4 $\mu$m) sarcomeres denoting fast fibers and long (>4 $\mu$m) sarcomeres denoting slow fibers. Fast and slow fibers occur in separate muscles in the abdomen of crayfish and lobsters and are used respectively for fast (escape) and slow (postural) movements. The cephalocarid abdominal muscle has sarcomeres of 5 $\mu$m, suggesting a slow fiber type; this is in keeping with the relatively slow movements of the abdomen. However, in this initial study of cephalocarid muscles, we cannot rule out the existence of fast type fibers with short sarcomeres in the abdomen.

Cephalocarid muscle deviates significantly from the general crustacean type in its lack of myofibrils. In the cephalocarids the muscle fiber remains as a single unit (which is punctuated with triads) (Fig. 4A), whereas in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most other crustaceans it is subdivided into numerous myofibrils (Fig. 4B, C) by elaboration of the sarcoplasmic reticulum, which forms an enveloping collar around each myofibril. In essence, these myofibrils functionally subdivide a single muscle fiber and permit a much finer control of its contraction. In the absence of myofibrils, such fine control may not accrue to the cephalocarid muscle, although the widespread distribution and high density of triads in the cephalocarid muscle fiber would ensure that no area of the fiber is far from these elements that couple excitation to contraction.

The only other known crustacean species in which the muscle fibers are also not subdivided into myofibrils by the sarcoplasmic reticulum is the ostracod *Cypridopsis vidua* (Fahrenbach, 1964) (Fig. 4A). Muscles of the appendages consist of single fibers that are the equivalent of single fibrils. Triads consisting of two elements of the sarcoplasmic reticulum and one of the t-tubule are usually scattered throughout these single fibers, although more complex associations, with three to nine elements of the sarcoplasmic reticulum, are also found. The latter associations denote a more elaborate development of the sarcoplasmic reticulum in the form of several layers of tubules. In comparison, the cephalocarid muscle with its triad denoting two tubules of the sarcoplasmic reticulum represents a less elaborate and much simpler system.

Muscle fibers in other known crustacean species are subdivided into myofibrils by a fenestrated collar of sarcoplasmic reticulum (Fig. 4C). At discrete locations around this collar are elements of the t-tubule giving rise to diads or triads. This type of organization is seen amongst all species examined in the class Malacostraca and these encompass stomatopods (*Hemisquilla:* McNeill *et al.*, 1972), amphipods (*Gammarus:* pers. obs.), and decapods (crabs, crayfish, and lobster: reviewed by Atwood, 1973). Amongst the other classes of crustacea, namely, Branchiopoda, Cephalocarida, Maxillopoda, Ostracoda, and Remipedia, only the last named has not been examined for its muscle fine structure. In Maxillopoda, myofibrils are seen in the barnacle *Balanus nubilus* (subclass Cirripedia) (Hoyle *et al.*, 1973) and in the copepod *Macrocyclops albidus* (subclass Copepoda) (Fahrenbach, 1963). In the latter case, diads and triads are found not only around the periphery of the myofibril (perifibrillar location) but also within the myofibril itself (intrafibrillar location) (Fig. 4B). In the class Branchiopoda, myofibrils—although poorly defined—are seen in *Daphnia*, with diads and triads restricted to the periphery of the myofibrils (pers. obs.). As mentioned above, only the ostracods and cephalocarids have muscles that lack myofibrils.

This brief comparative survey demonstrates a range of conditions from a relatively simple system of tubules of sarcoplasmic reticulum to a complex system that subdivides a muscle fiber into myofibrils. Triads found scattered over the entire muscle fiber in the case of the simple condition are restricted to the periphery of the myofibril in the more complex condition. Accordingly, ostracod and cephalocarid muscles with scattered triads (Fig. 4A) represent an early stage; copepod muscle represents an intermediate stage, with triads located both within the myofibril and along its periphery (Fig. 4B); and cirripeds, branchiopods, and malacostracans represent a more advanced stage, with the triads restricted to the periphery of the myofibrils (Fig. 4C).

While the lack of myofibrils represents a simple condition in the striated muscle organization of cephalocarids, does it also signify a primitive condition? Comparison with an outgroup such as the Onychophora throws light on this question. Muscle fibers in the onycophoran *Peripatus dominicae* are not subdivided into myofibrils, although the fiber is populated with many tubule systems, including sarcoplasmic reticulum and t-tubules (Hoyle and Williams, 1980). As a result, diads and triads occur scattered over the muscle fiber as in the cephalocarid muscle. Based on the view that the onychophorans predate the crustaceans, the lack of myofibrils may be construed as a primitive feature.

### *Neuromuscular terminals*

The fine structure of motor nerve terminals in the abdominal muscles of the cephalocarid is similar to that seen in the decapods (Govind and Atwood, 1982). However, although both excitatory and inhibitory motor nerve terminals are readily seen in the abdominal muscles of

**Figure 1.** A. Cross-section of the cephalocarid ventral abdominal longitudinal muscle showing a single layer of fibers (asterisks) cut at various planes, such as through the A-band (A), I-band (I), and Z-line (Z). Muscle nuclei (N) and mitochondria are restricted to the edge of the band, and the muscle fibers lack myofibrils. Nerve terminals (T) are located amongst muscle granular sarcoplasm (S). B. Cross-section showing thick and thin filaments and triads (arrows) composed of a long, narrow t-tubule element flanked on either side by cisternae of the sarcoplasmic reticulum. C. Longitudinal section showing well-defined sarcomeres between adjacent Z-lines (Z), on either side of which are I-bands (I) enclosing a central A-band (A). Elements of sarcoplasmic reticulum are seen as longitudinal tubules (arrowhead). Magnifications: ×5400 (A), ×96,000 (B), ×10,300 (C). Scale bars: 5 μm (A), 0.25 μm (B), 3 μm (C).

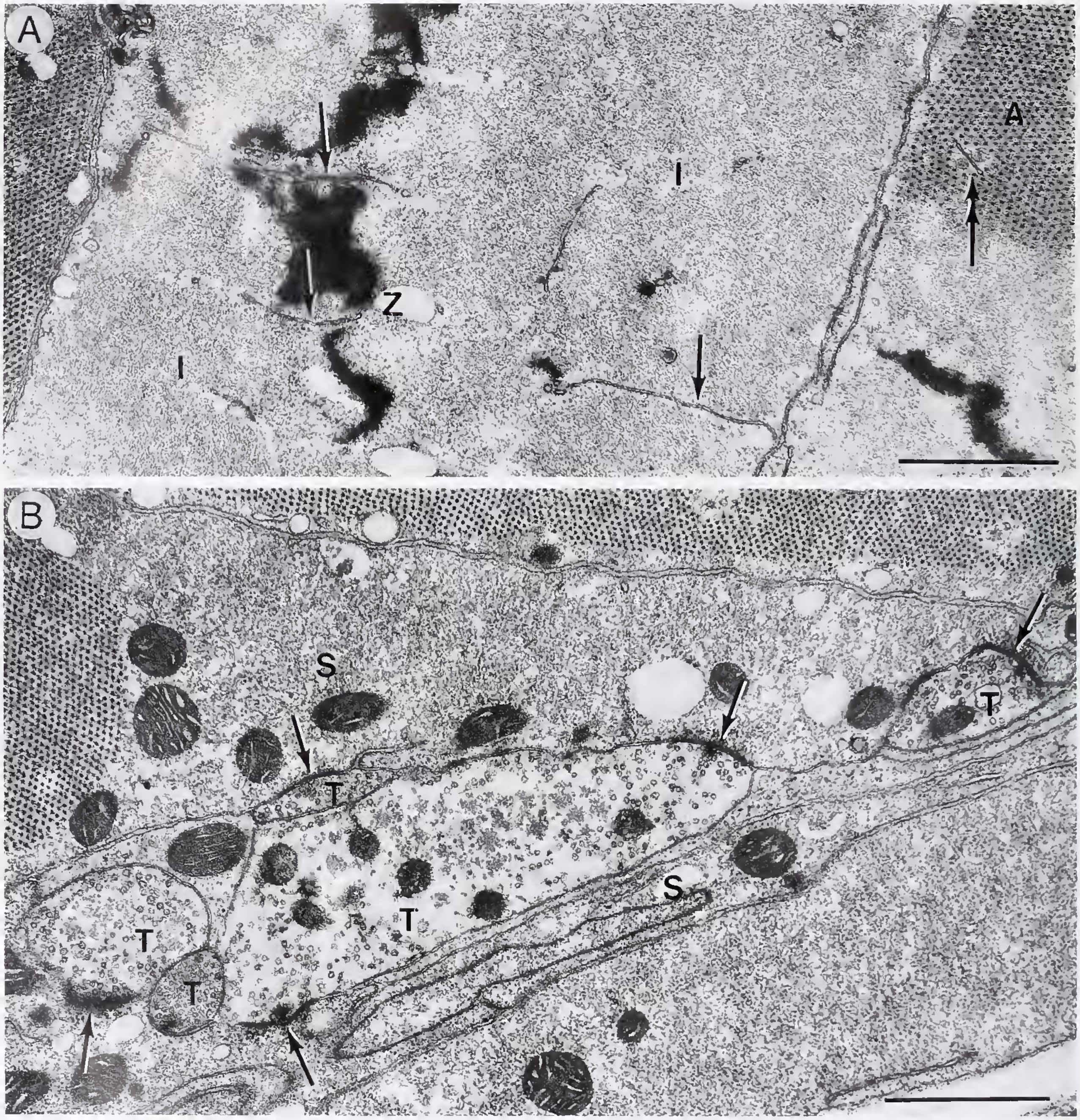


Figure 2. A. Cross-section of a ventral abdominal muscle through the A-band, I-band (I), and Z-line (Z) showing t-tubules through the I-band, Z-line (arrows), and the A-band (double arrow) where it forms part of a triad. B. Cross-section showing five variously sized profiles of nerve terminals (T) embedded in muscle granular sarcoplasm (S). Terminals are recognized by aggregations of clear synaptic vesicles that are spherical in shape. Synaptic contacts (arrows) are recognized by dense staining of regularly aligned presynaptic and postsynaptic membranes. At some of these contacts the presynaptic side has a dense body representing an active zone. Magnification: ×27,000. Scale bars: 1 $\mu$m.

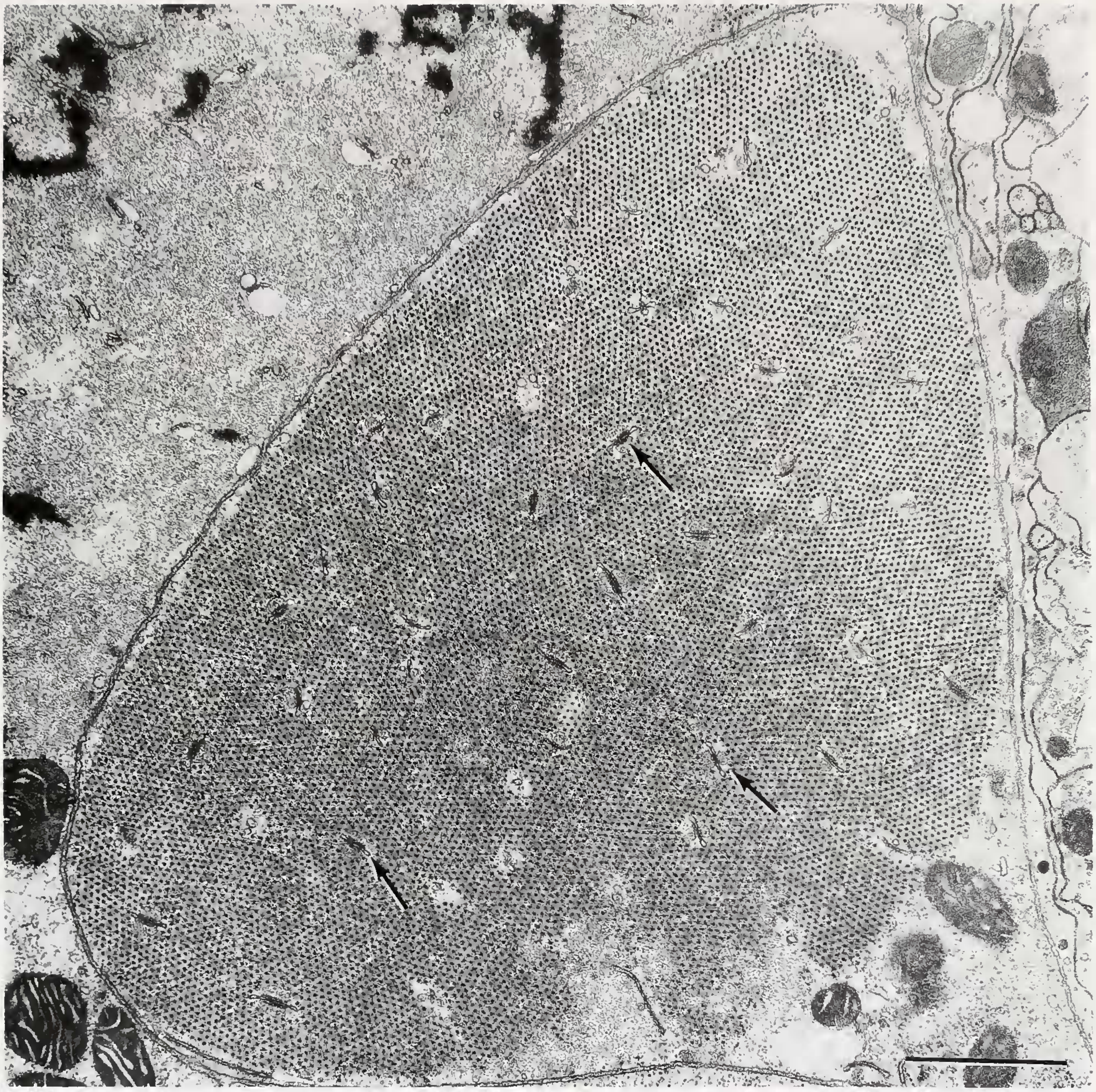

**Figure 3.** Cross-section of a single ventral abdominal muscle fiber through the A-band region packed with myofilaments and numerous scattered triads (arrows), but not subdivided into myofibrils. Note peripherally located mitochondria. Magnification: ×27,000. Scale bar: 1 μm.

tailed decapods such as crayfish and lobsters, the cephalocarid abdominal muscles displayed terminals with round synaptic vesicles, suggesting only excitatory motor innervation. Such a conclusion, although tentative, is in keeping with the relatively simple organization of the abdominal muscles that generate largely flexion-type movements and occur as a sheath lining the abdominal cylinder.

Simplicity in the neuromuscular terminals of the cephalocarid abdomen is also seen in the minimal elaboration of the surrounding muscle granular sarcoplasm. Thus synaptic contacts with the muscle membrane are made very close to the myofilaments, with little intervening sarcoplasm. In contrast, neuromuscular synaptic contacts occur on a highly elaborated sarcoplasmic reticulum

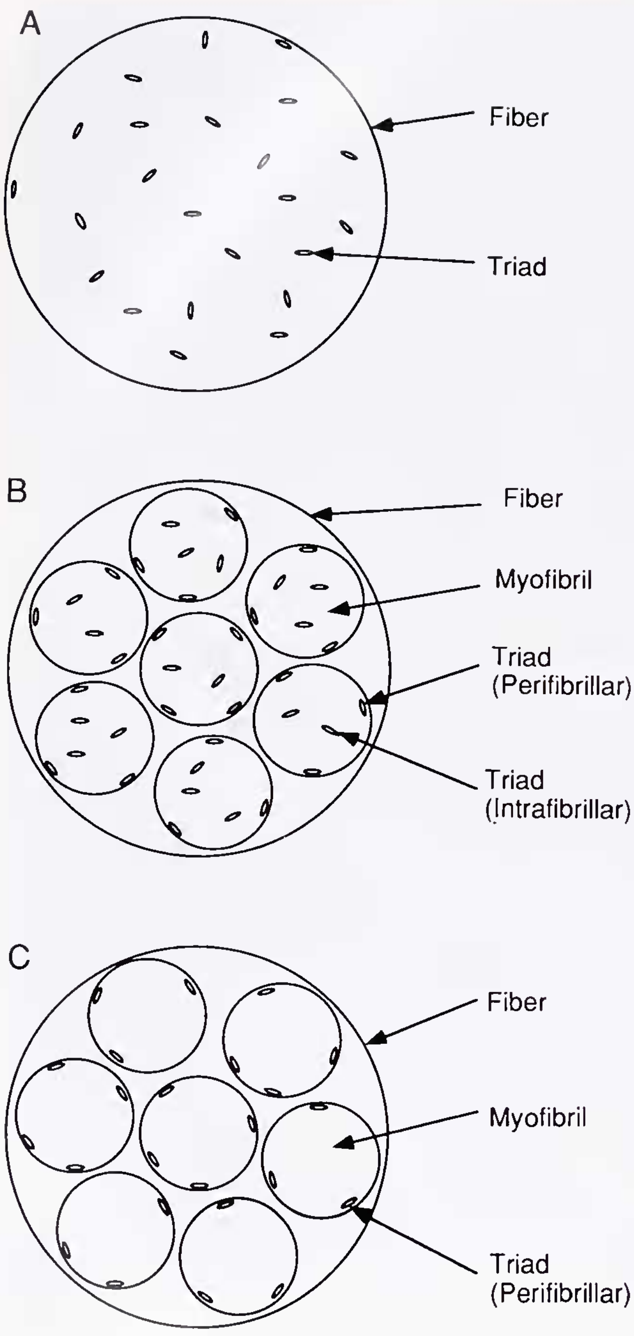


**Figure 4.** Stylized cross-sectional views of single muscle fibers amongst crustaceans, showing distribution of triads and subdivision into myofibrils. A. Single fiber not subdivided into myofibrils and with scattered triads as in the ostracod *Cypridopsis vidua* (Fahrenbach, 1964) and the cephalocarid *Hutchinsoniella macracantha* (present report). B. Single fiber subdivided into myofibrils by longitudinally running sarcoplasmic reticulum and with triads located along the periphery of the myofibril (perifibrillar location) as well as within the myofibril (intrafibrillar location), as in the copepod *Macrocyclops albidus* (Fahrenbach, 1963). C. Single fiber subdivided into myofibrils by sarcoplasmic reticulum and with triads located along the periphery of the myofibril (perifibrillar location), as in the majority of crustaceans, including stomatopods, amphipods, and decapods.

amongst the decapods (Atwood, 1976; Govind and Atwood, 1982), reflecting a more extensive repertoire of movements than that of the cephalocarids.

## Acknowledgments

We are indebted to Arthur Humes for facilitating this study and to Joanne Pearce and two anonymous reviewers for a critical reading of the manuscript. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada to C.K.G. and by the U.M. EgenFicka Fund to R.R.H.

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